CLAIMS

We Claim:

1. A photocleavable compound having the general formula:

A - L - PR - AL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group, AL is a second linker comprising one or more $-H_2C-NH-$ groups, wherein said second linker is labeled with one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins.

2. The compound of Claim 1, wherein said second linker comprises a structure of the general formula:

$$-HN-(CH_2)_n-NH-$$

wherein n is a whole number between 1 and 10.

3. The compound of Claim 1, wherein said second linker comprises a structure derived from a diamine, wherein said diamine is selected from the group consisting of 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane, 1,7-diaminoheptane, 1,8-diaminooctane and 2,2'-(Ethylenedioxy)-diethylamine.

4. The compound of Claim 1, wherein said second linker comprises a structure of the general formula:

$$-HN-(CH_2-CH_2-O)_n-CH_2-CH_2-NH-$$

wherein n is a whole number between 1 and 10.

5. The compound of Claim 1, wherein said affinity moiety comprises biotin.

6. The compound of Claim 1, wherein said protein reactive group is a sulfhydryl reactive group.

7. The compound of Claim 1, wherein said protein reactive group is an amine reactive group.

8. A photocleavable compound having the general formula:

A - L - PR - AAL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group, AAL is a second linker comprising a structure derived from an amino acid, said second linker labeled with one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins.

9. The compound of Claim 8, wherein said second linker comprises a structure derived from an amino acid selected from the group consisting of valine, leucine, and isoleucine, wherein said structure is linked to said photocleavable group through an α-amino group and linked to said protein reactive group through an α-carboxyl group.

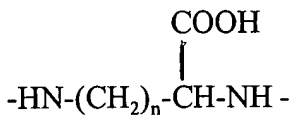
10. The compound of Claim 8, wherein said second linker comprises a structure derived from an amino acid selected from the group consisting of valine, leucine, and isoleucine, wherein said structure is linked to said protein reactive group

through an α-amino group and linked to said photocleavable group through an α-carboxyl group.

11. The compound of Claim 8, wherein said second linker comprises a structure derived from lysine, wherein said structure is linked to said photocleavable group through an ϵ-amino group and linked to said protein reactive group through an α-amino group.

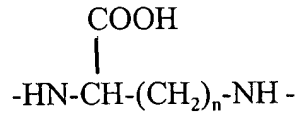
12. The compound of Claim 8, wherein said second linker comprises a structure derived from lysine, wherein said amino acid structure is linked to said protein reactive group through an ϵ-amino group and linked to said photocleavable group through an α-amino group.

13. The compound of Claim 8, wherein said second linker is of the general formula



wherein n is a whole number between 1 and 10.

14. The compound of Claim 8, wherein said second linker is of the general formula



wherein n is a whole number between 1 and 10.

15. The compound of Claim 8, wherein said protein reactive group is a sulfhydryl reactive group or an amine reactive group.

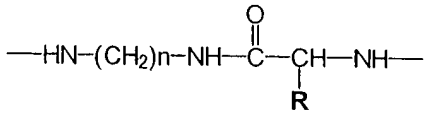
16. A photocleavable compound having the general formula:

A - L - PR - AAL - AL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group; AAL is a second linker comprising a structure derived from an amino acid, said second linker labeled with one or more stable isotopes; AL is a third linker comprising one or more $-H_2C-NH-$ groups; and PRG is a protein reactive group that reacts

with functional groups on proteins.

17. The compound of Claim 16, wherein the AAL-AL portion is of the general formula:

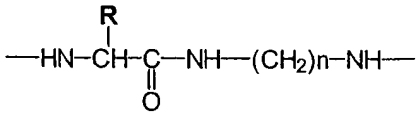


18. A photocleavable compound having the general formula:

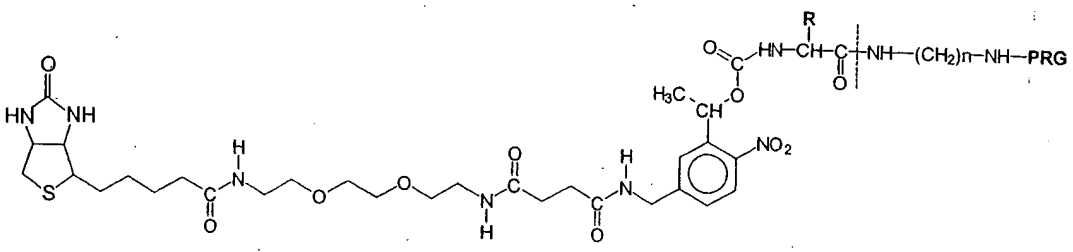
A - L - PR - AL - AAL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group; AL is a second linker comprising one or more -H_2C-NH- groups; AAL is third linker comprising a structure derived from an amino acid, said structure labeled with one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins.

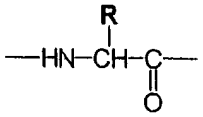
19. The compound of Claim 18, wherein the AL-AAL portion is of the general formula:



20. A photocleavable compound having the general formula:



wherein R is an aliphatic hydrocarbon chain; wherein the portion defined by



comprises one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins.

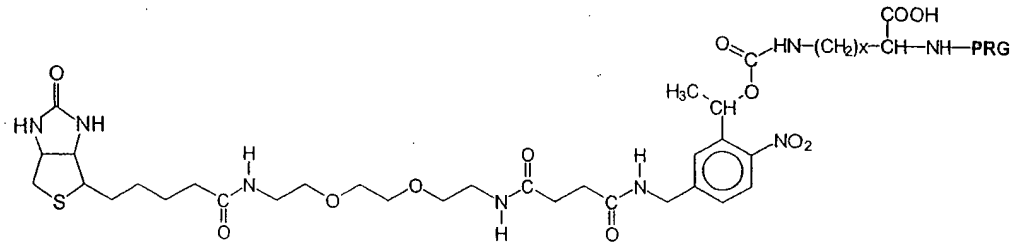
21. The compound of Claim 20, wherein said protein reactive group is a sulfhydryl

reactive group or an amine reactive group.

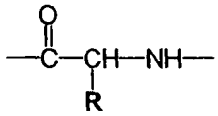
22. The compound of Claim 20, wherein said aliphatic hydrocarbon chain is greater than two carbons in length.

23. The compound of Claim 20, wherein said aliphatic hydrocarbon chain is branched.

24. A photocleavable compound having the general formula:



wherein R is an aliphatic hydrocarbon chain; wherein the portion defined by



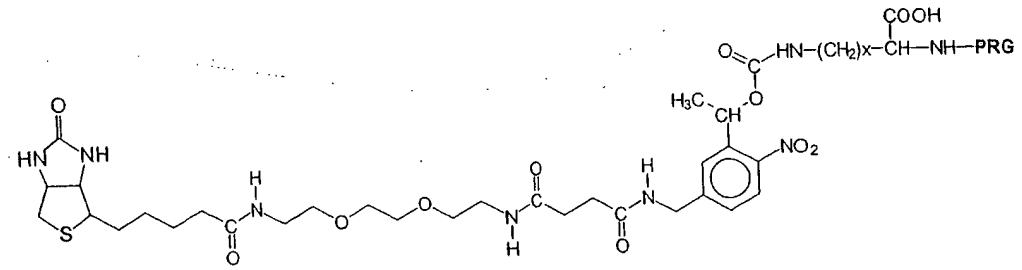
comprises one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins.

25. The compound of Claim 24, wherein said protein reactive group is a sulfhydryl reactive group or an amine reactive group.

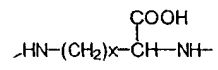
26. The compound of Claim 24, wherein said aliphatic hydrocarbon chain is greater than two carbons in length.

27. The compound of Claim 24, wherein said aliphatic hydrocarbon chain is branched.

28. A photocleavable compound having the general formula:



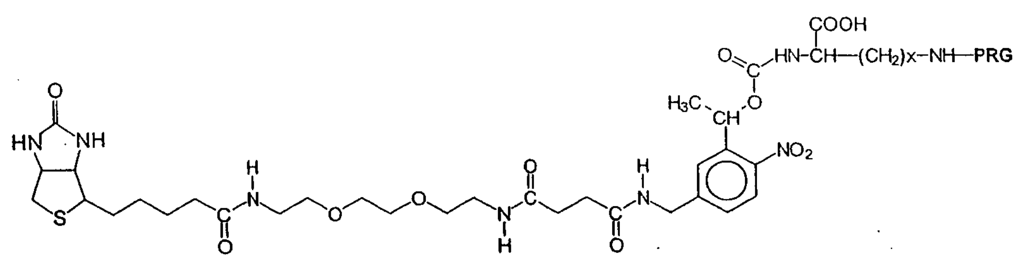
wherein x is a whole number between 1 and 10; wherein the portion defined by



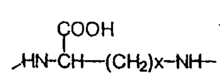
comprises one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins.

29. The compound of Claim 28, wherein said protein reactive group is a sulfhydryl reactive group or an amine reactive group.

30. A photocleavable compound having the general formula:



wherein x is a whole number between 1 and 10; wherein the portion defined by



comprises one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins.

31. A method, comprising:

a) providing i) a diamine; and ii) PC-Biotin-NHS; and

b) reacting said diamine with said PC-Biotin-NHS under conditions such that a first product is generated of the general formula:

Biotin-L-PR-AL

wherein L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group, and AL is a second linker comprising one or more $-H_2C-NH-$ groups.

32. The method of Claim 31, further comprising c) reacting said first product with a compound comprising an amino acid structure so as to generate a second product of the general formula:

Biotin-L-PR-AL-AAL

wherein AAL is a third linker comprising a structure derived from an amino acid.

33. The method of Claim 31, wherein said second linker is labeled with one or more stable isotopes.

34. The method of Claim 32, wherein said third linker is labeled with one or more stable isotopes.

35. The method of Claim 31, wherein said diamine structure is of the general formula:

$$H_2N\text{-}(CH_2)_n\text{-}NH_2$$

36. The method of Claim 31, wherein said diamine is selected from the group consisting of 1,3-diaminopropane, 1,4-diaminobutane, 1,5-diaminopentane, 1,6-diaminohexane 1,7-diaminoheptane, 1,8-diaminooctane and 2,2'-(Ethylenedioxy)diethylamine.

37. The method of Claim 31, wherein said diamine is of the general formula:

$$H_2N\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2\text{-}NH_2$$

wherein n is a whole number between 1 and 10.

38. The method of Claim 31, wherein said PC-Biotin-NHS compound is selected from the group of compounds shown in Figure 1.

39. The method of Claim 32, wherein the AL-AAL portion is of the general formula:

O R

-HN-$(CH_2)_n$-NH-C-CH-NH

40. A method, comprising:

a) providing i) a compound comprising an amino acid structure, and ii) PC-Biotin-NHS; and

b) reacting said compound with said PC-Biotin-NHS under conditions such that a first product is generated of the general formula:

Biotin-L-PR-AAL

wherein L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group, and AAL is a second linker comprising a structure derived from an amino acid.

41. The method of Claim 40, further comprising c) reacting said first product with a diamine so as to generate a second product of the general formula:

Biotin-L-PR-AAL-AL

wherein AL is a third linker comprising one or more $-H_2C-NH-$ groups.

42. The method of Claim 40, wherein said second linker is labeled with one or more stable isotopes.

43. The method of Claim 41, wherein said third linker is labeled with one or more stable isotopes.

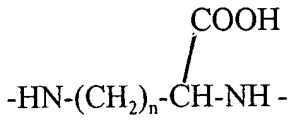
44. The method of Claim 40, wherein said compound is selected from the group consisting of valine, leucine, and isoleucine, wherein said amino acid structure is linked to said photocleavable group through an α-amino group and linked to said protein reactive group through an α-carboxyl group.

45. The method of Claim 40, wherein said compound is selected from the group consisting of valine, leucine, and isoleucine, wherein said amino acid structure is linked to said protein reactive group through an α-amino group and linked to said photocleavable group through an α-carboxyl group.

46. The method of Claim 40, wherein said compound comprises lysine, wherein said amino acid structure is linked to said photocleavable group through an ϵ-amino group and linked to said protein reactive group through an α-amino group.

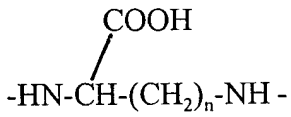
47. The method of Claim 40, wherein said compound comprises lysine, wherein said amino acid structure is linked to said protein reactive group through an ϵ-amino group and linked to said photocleavable group through an α-amino group.

48. The method of Claim 40, wherein said second linker is of the general formula



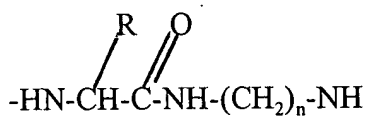
wherein n is a whole number between 1 and 10.

49. The method of Claim 40, wherein said second linker is of the general formula



wherein n is a whole number between 1 and 10.

50. The method of Claim 41, wherein the AAL-AL portion is of the general formula:



wherein n is a whole number between 1 and 10.

51. A method, comprising:

a) providing i) a mixture comprising proteins, ii) a solid support, and iii) a photocleavable compound of a general formula selected from the group consisting of:

A - L - PR - AL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group, AL is a second linker comprising one or more $-H_2C-NH-$ groups, wherein said second linker is labeled with one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins;

A - L - PR - AAL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group, AAL is a second linker comprising a structure derived from an amino acid, said second linker labeled with one or more stable isotopes;

and PRG is a protein reactive group that reacts with functional groups on proteins;

A - L - PR - AAL - AL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group; AAL is a second linker comprising a structure derived from an amino acid, said second linker labeled with one or more stable isotopes; AL is a third linker comprising one or more $-H_2C-NH-$ groups; and PRG is a protein reactive group that reacts with functional groups on proteins; and

A - L - PR - AL - AAL - PRG

wherein A is an affinity moiety that binds to a capture reagent; L is a first linker that is not labeled with stable isotopes; PR is a photocleavable group; AL is a second linker comprising one or more $-H_2C-NH-$ groups; AAL is third linker comprising a structure derived from an amino acid, said structure labeled with one or more stable isotopes; and PRG is a protein reactive group that reacts with functional groups on proteins;

b) contacting said mixture with said photocleavable compound under conditions such that a population of protein-photocleavable compound conjugates is created;

c) subjecting at least a portion of said population to proteolysis so as to create a plurality of peptide-photocleavable compound conjugates;

d) capturing at least a portion of said plurality of peptide-photocleavable compound conjugates on said solid support to create immobilized conjugates; and

e) exposing said immobilized conjugates to electromagnetic radiation under conditions such that at least a portion of said immobilized conjugates is released from said solid support so as to create released peptides.

52. The method of Claim 51, further comprising f) analyzing said released peptides using mass spectrometry.

53. The method of Claim 51, wherein said stable isotopes are selected from the ^{2}H, ^{13}C, and ^{15}N.